

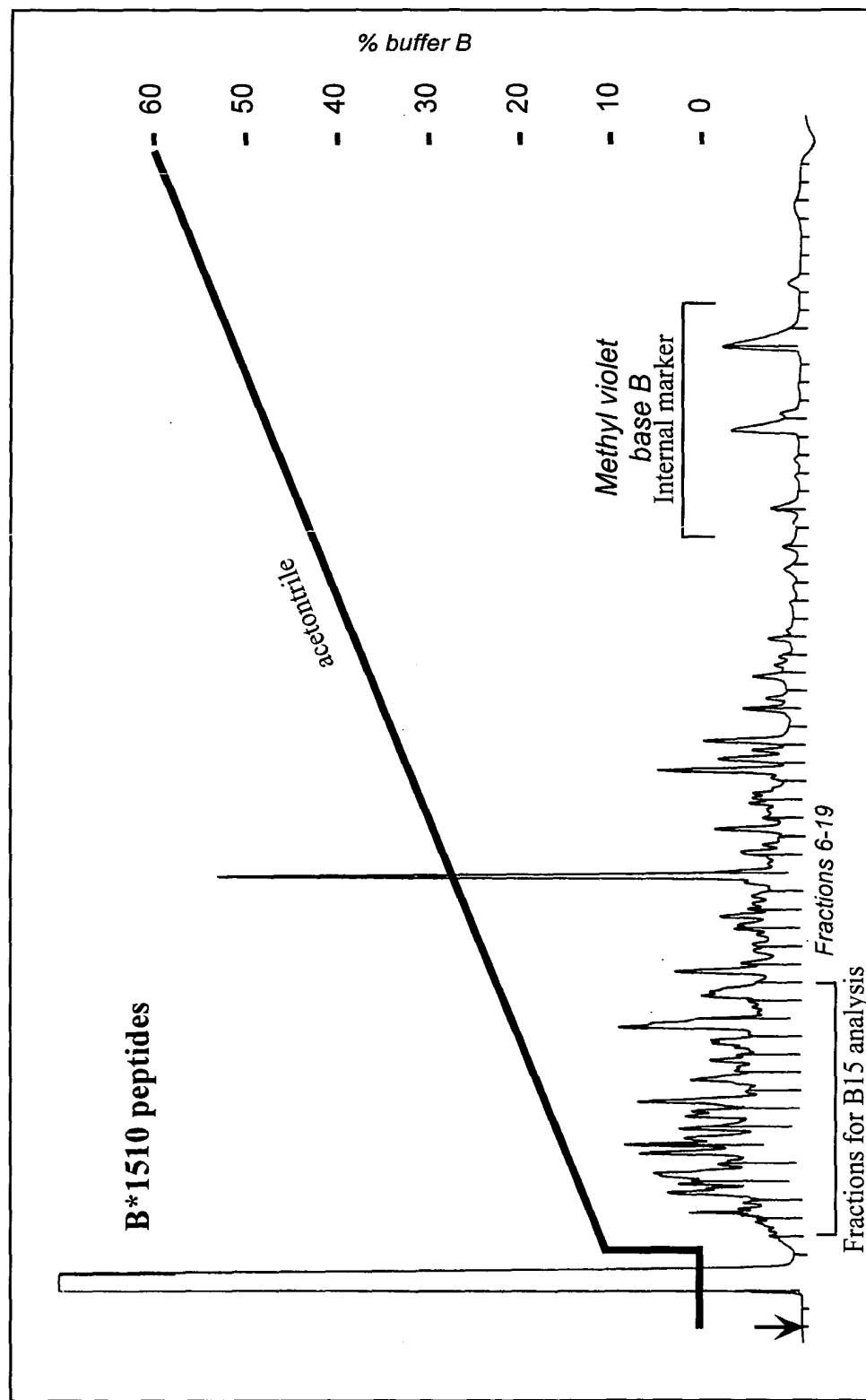
Title: **SOLUBLE HLA LIGAND DATABASE UTILIZING
PREDICTIVE ALGORITHMS AND METHODS OF MAKING
AND USING SAME**

Applicant: **William Hildebrand**
Filed: **02/21/2002**
Atty: **Douglas J. Sorocco**

Group: **2171**
Examiner: **Unknown**
Tel: (405) 478-5344

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Reverse phase HPLC of class I HLA eluted peptide ligands



Fraction Number

Fig. 1

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Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

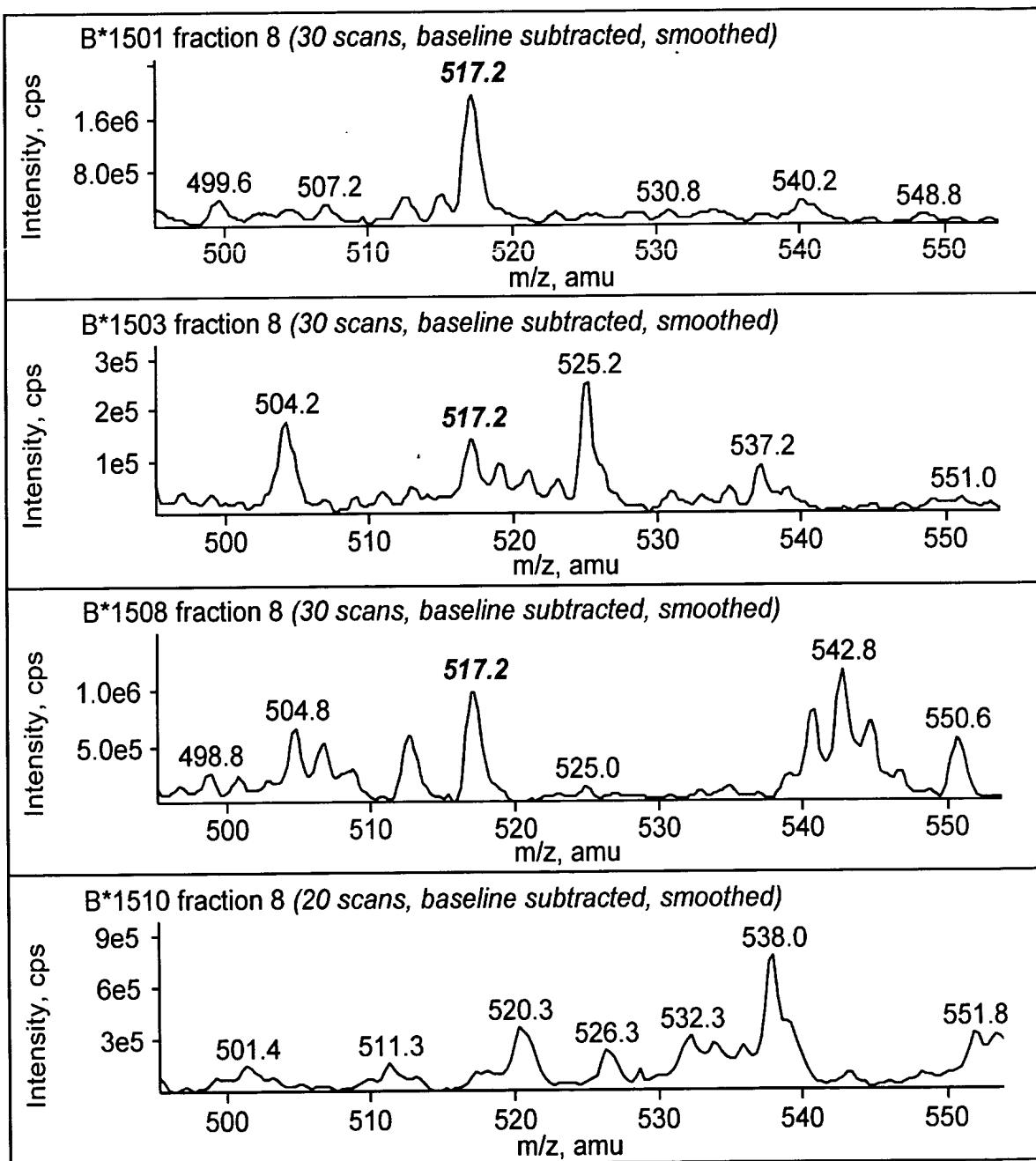


Fig. 2

Serial No. 10/082,034 Dkt. No. 6680.040
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20250707 11230007
 MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I
 sHLA molecules. This data was accomplished by completing a second
 nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how
 ions can be MS ion mapped and subsequently MS/MS sequenced. There is
 sufficient peptide present to do multiple MS/MS fragmentation runs. There is
 also sufficient peptide present to facilitate a submotif on fraction 8 or further
 separation in the event that two peptides had mapped at 517.2 in the ion map.

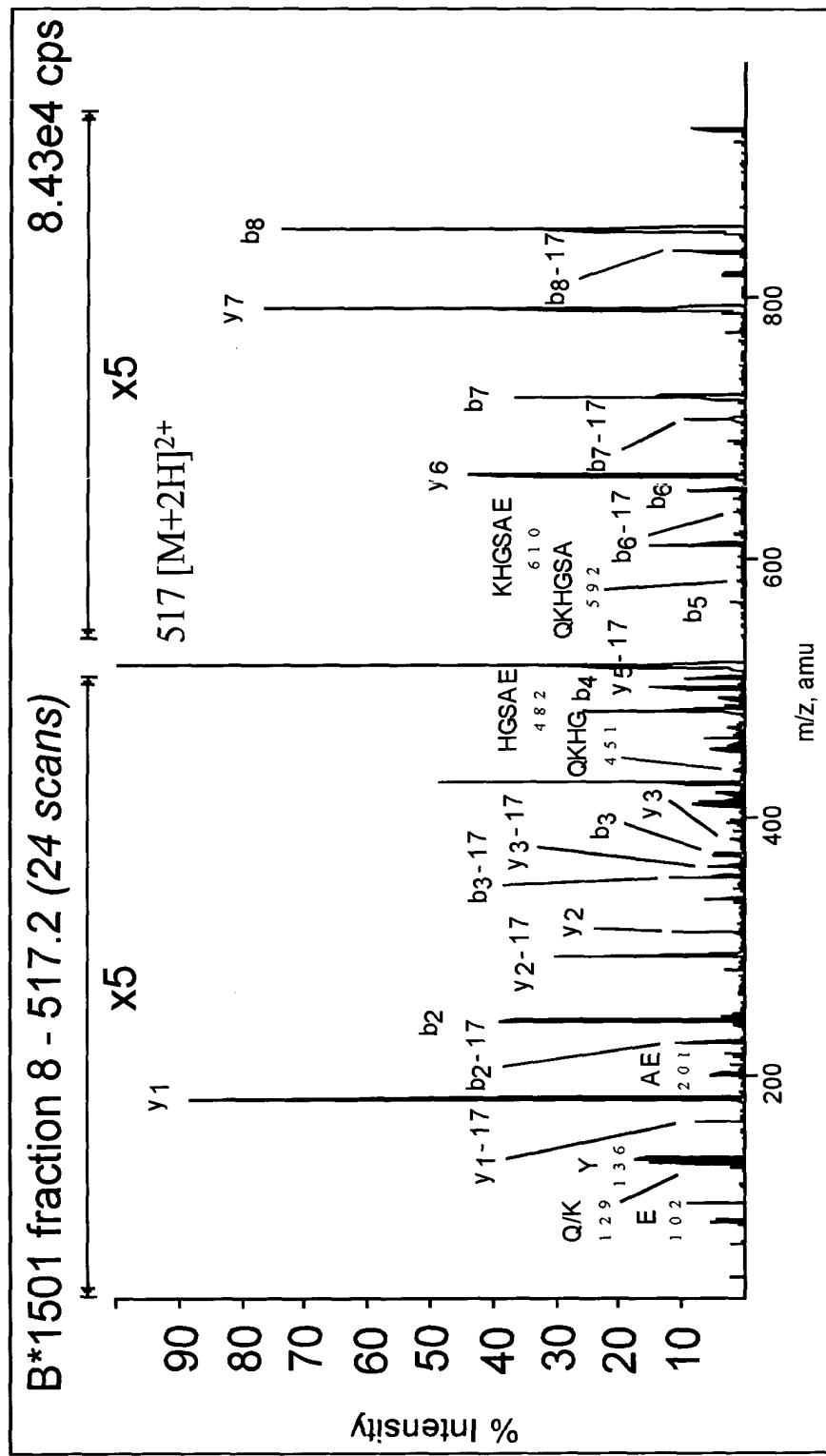


Fig. 3 1 of 3

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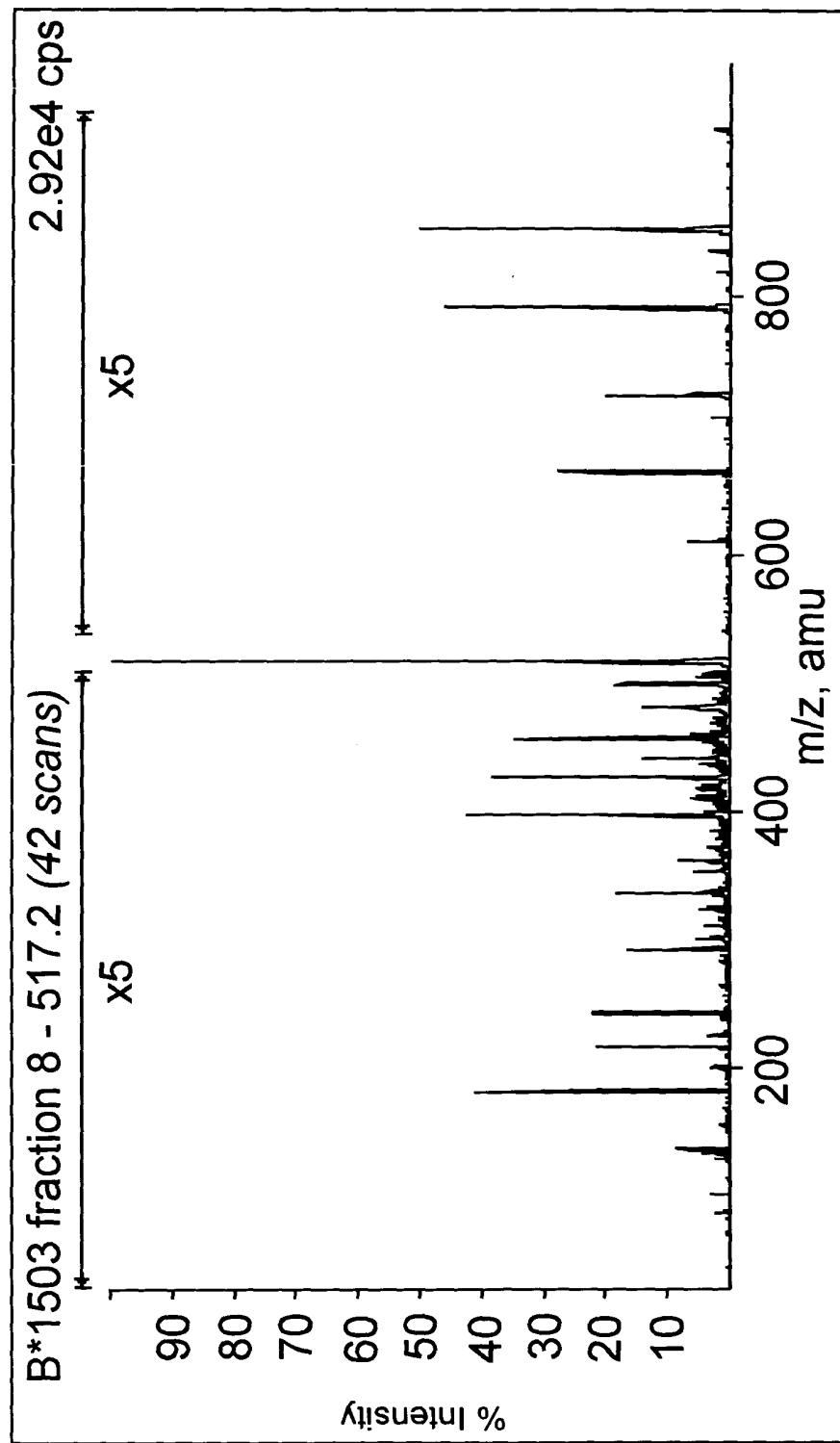


Fig. 3 2 of 3

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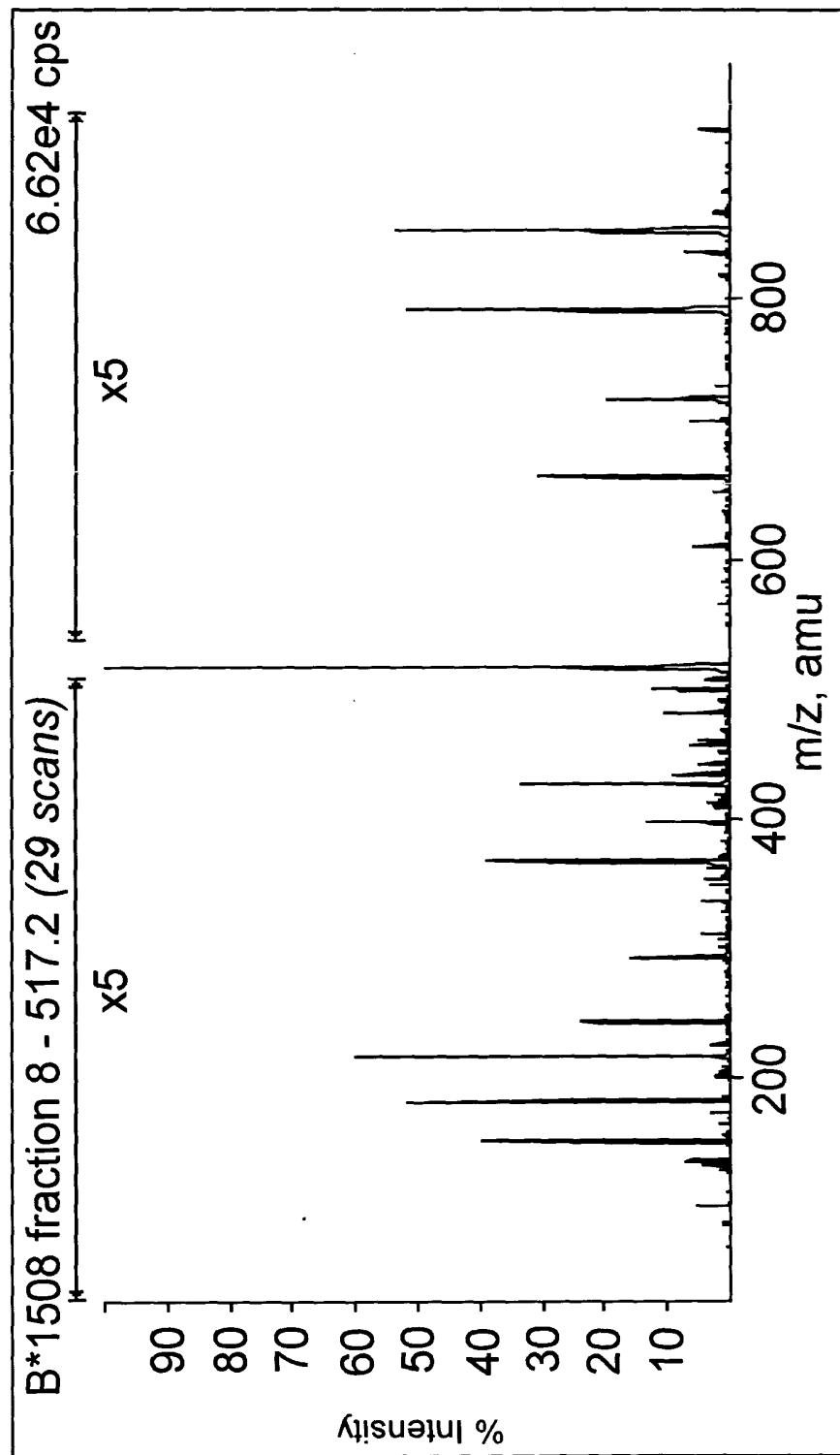
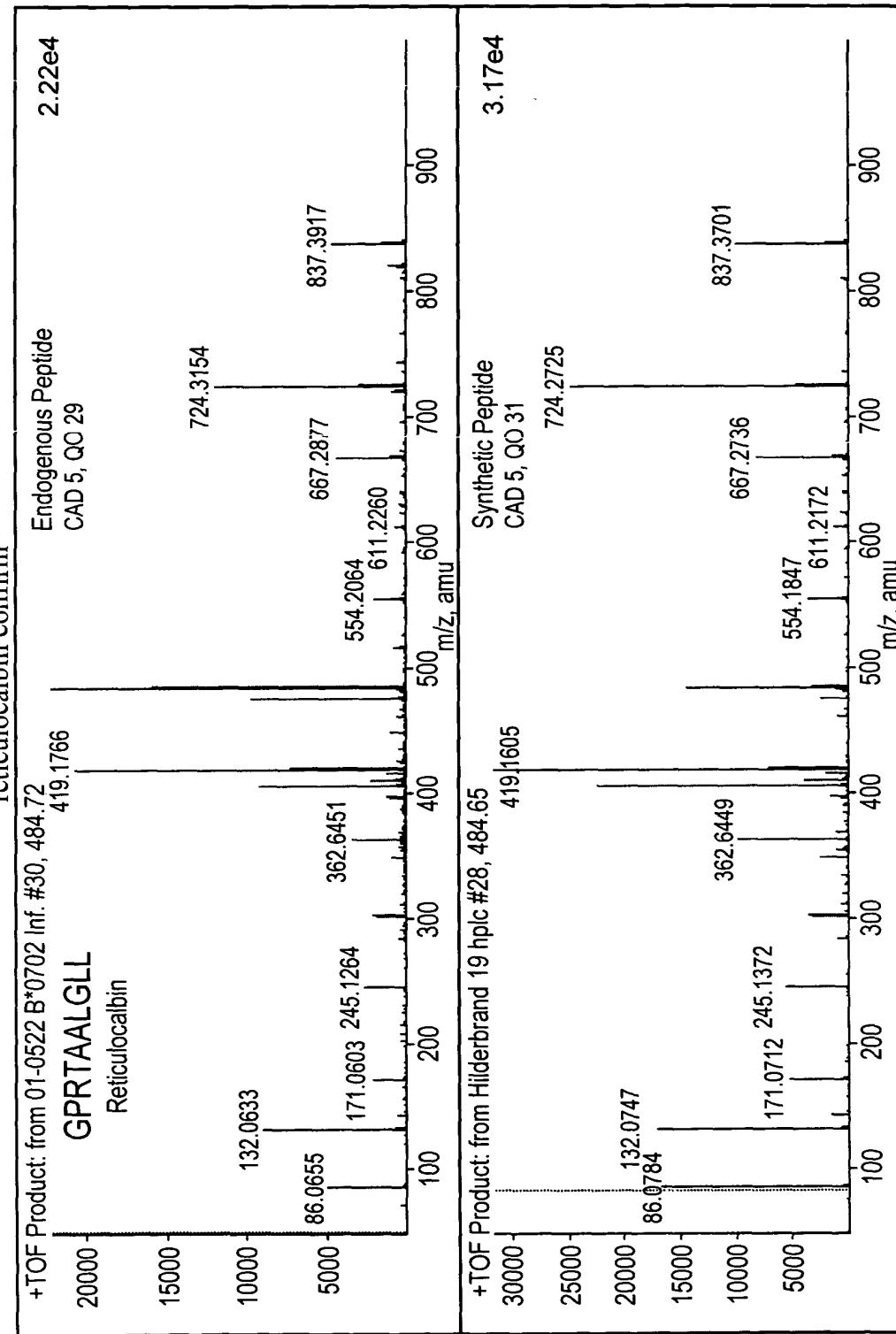


Fig. 3 3 of 3

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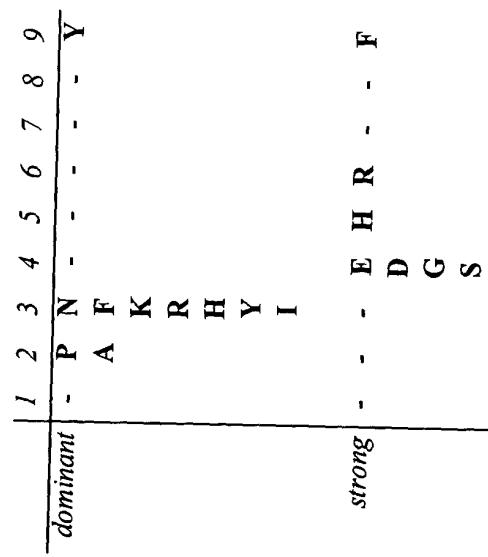
sHLA B*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

Fig. 4

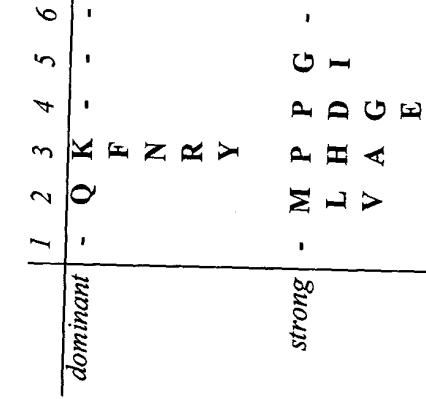
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B*1508



B*1501



B*1510

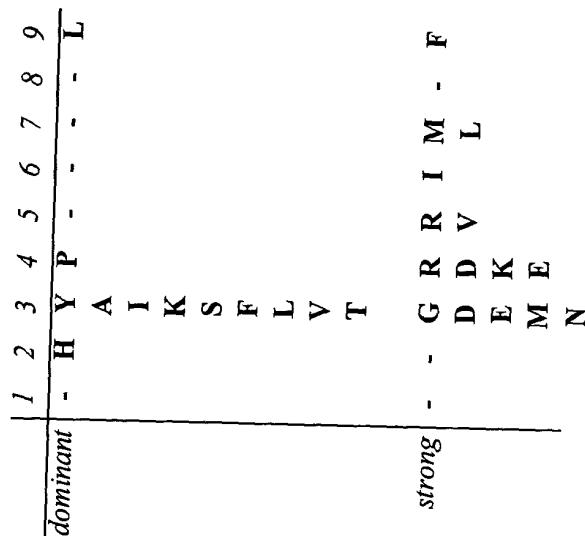


Fig. 5

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--WDRHTXF AQYAAAGESFY ILGPPGSVY
 ---YT TPHTZHDEY XIGDVNMVY
 XFAZEHTTY AP---XVSY
 NGXAMHWTY MVGXXPAT YMSGXYGTF
 VPCGZZSY DPHYVSGHZF --AVVAZSY
 TAZZHRGY VVACV--Y MPAGYNNVY
 NOZHGSAY PLA-N-HTY XEVVPAAZSY
 TG---AY VVAPITRGY YMIDPSGSVY
 NGYDGPNAQY PXAMOXYTY
 TPXGEPYZSY -P-MPGXAY
 FVSNHAY TZSNAY
 SOFGGGSQY MAAMVGAVY
 SQFDHVTY LPHQPLAVY
 XAN--VT FVTXNKEEY
 VDX--Y GPZVMZHG
 CPLSCFT FOARXTEY
 FLZAMZSTY AAXV--VTV
 TPEMGSZTHY XPEMGSFSY
 DPSGTZSGF YV--VR-VF
 ---PGFY AAPVGAXESY
 -A-PHPMGY YVA---PAF
 AQTVGYGEY VGY--AHPGF
 ---SVY ---STY
 TGNCSGTGTY SPTYTHAVAF
 AQVNPSXTY MPA--MVMAF
 TP--ARAPT XA---SYTY
 SPGAETRAXY VGYVDDTQF
 YX---RTF ZATNSVTSTY
 YKG---GAF YATAGEMMAF
 -P---PSSGY TARVXSVY
 TP---GRMY MPAADYEVAF
 PMFDZZVY AAFCG--XV
 AQEHGCAZF SPNEDXMZVF
 -M---GVHDI VAATAGAVF
 YVS--RNZY XLH--ET

--WDRHTXF FLZAMGSTY YMVT---F
 ---YT GOYVZPPTY APAV---VGY
 ALGA---RGY PMDPZPTF ---TGF
 XS---VEY XAVGHSGGTY PVPNVRXNY
 AQFASGAGZ ---PTY ---TXSX
 -G---CDY ---PSY YMVCNAEY
 ---ZARGY EPAMVXZCF ---XRDXY
 ALNGRVTMY XAHTECPRGY SLX---F
 DPHAPPZY VOGPGVZY ALGSZAXMPF
 XAZVZMTAY TGAPVSEEGY VGYVDDTQF
 NOZHGSAY VOXYYGSVY DVEGWMMSZY
 FGXACXATSY GPGAPXGGZ YQHPSAXRF
 APMARQZY GPHNGXRAY ---ZAY
 TG---AY AAHWHVEAY GLGZTSAEF
 GOZZAVDF TPTRRESY NAXG--RESSF
 TPXGEPYZSY FPTDRRSZF TARVXSVY
 GOHASVXS YTGVSYXHF AAFCG---XV
 FVSNHAY AQASAPDAY XLH--ET
 ---SYGORKGAGSFSV ILGPPGSVY
 NPAZZPN VOYXPF XLDGVNNY
 -O-DPPPDMZYATGTAZNNXNZYVMGXTNANF
 XQ---AGGZY VVACV---Y
 SOFGGGSQY PLA-N-HTY AMNPTNTVDF
 SQFDHVTY VVAPITRGY SOXAAGDVDF
 -AAHVPPGY XQYTVGY -VFSHTTF
 FMDVGAFTVY PLFGZTAGZY XOGHHEMFY
 XAN--VTF POGZMA--Y
 AQM--SEY VAGGW ---ZXEY
 CPLSCFT SGAXDRAYZF YMIDPSGSVY
 FLZAMZSTY VQGPVGTDF
 TVXDSZTHY FOARXTEY
 AQAAPEFAGY XAGFFXXEY
 ---FGHY XQ---ZY
 ALW---PZF EO---TX
 VPHZNAY TP---AZAF
 ---GHGGY VVATZNZZX

Fig. 5 continued

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Pooled Peptide
Motif

P1 P2 P3 P4 P5 P6 P7 P8 P9
T R P
S E
Q M Y K D H

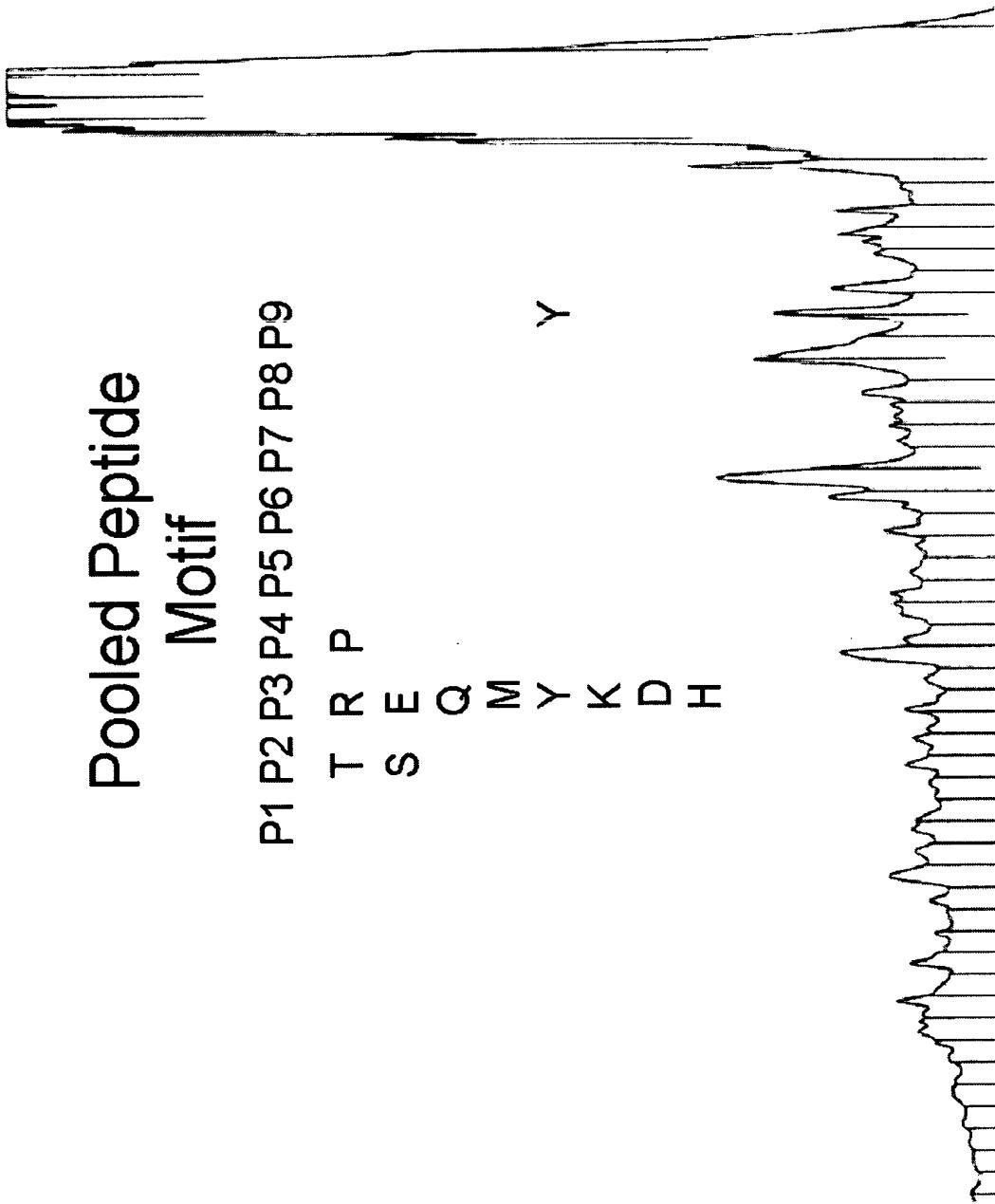


Fig. 6

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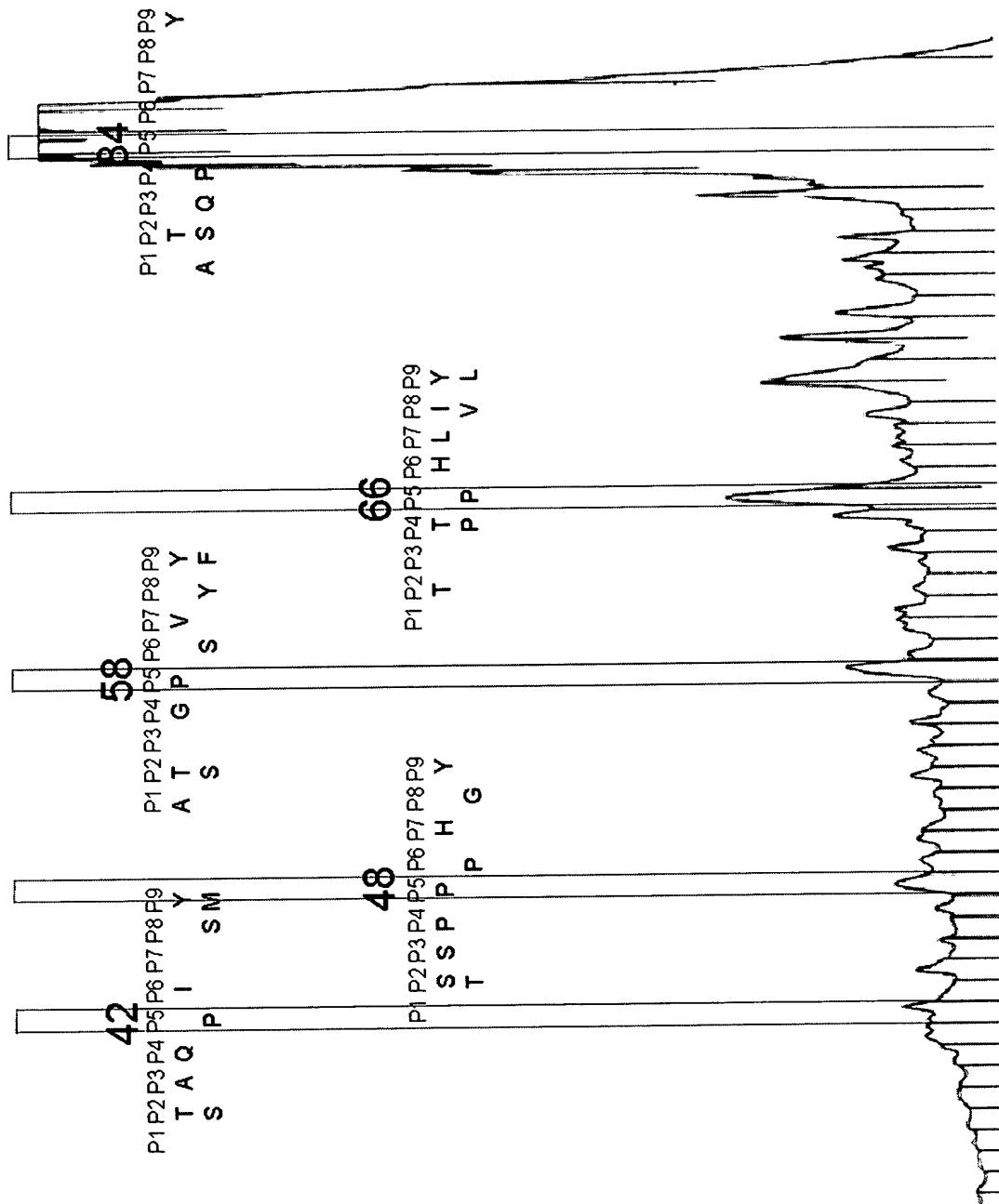


Fig. 7

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Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEYPRPIPH	IGPDYIPTEE	ERRYFAECND
AATSMUTQG	LISKGILSSH	PKYGSPKLI	LACMGYFAG
KFKKLENSPL	GEALRSGQAR	RSSPPGHYYQ	KSKYDSSVSG
QSSFVTPSAA	DNIEMLPHYE	PIPPSSSMNE	SAPTGITDHI
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERYP
DTwDE			

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEYPRPIPH	IGPDYIPTEE	ERRYFAECND
AATSMUTQG	LISKGILSSH	PKYGSPKLI	LACMGYFAG
KFKKLENSPL	GEALRSGQAR	RSSPPGHYYQ	KSKYDSSVSG
QSSFVTPSAA	DNIEMLPHYE	PIPPSSSMNE	SAPTGITDHI
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERYP
DTwDE			

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

ESFWFRSYPL	KLSYYVKTQEQ
QSSFVTPSAA	YQGPDPNLEE
RKEVKNKYG	KKEYVKNKYG

Fig. 8

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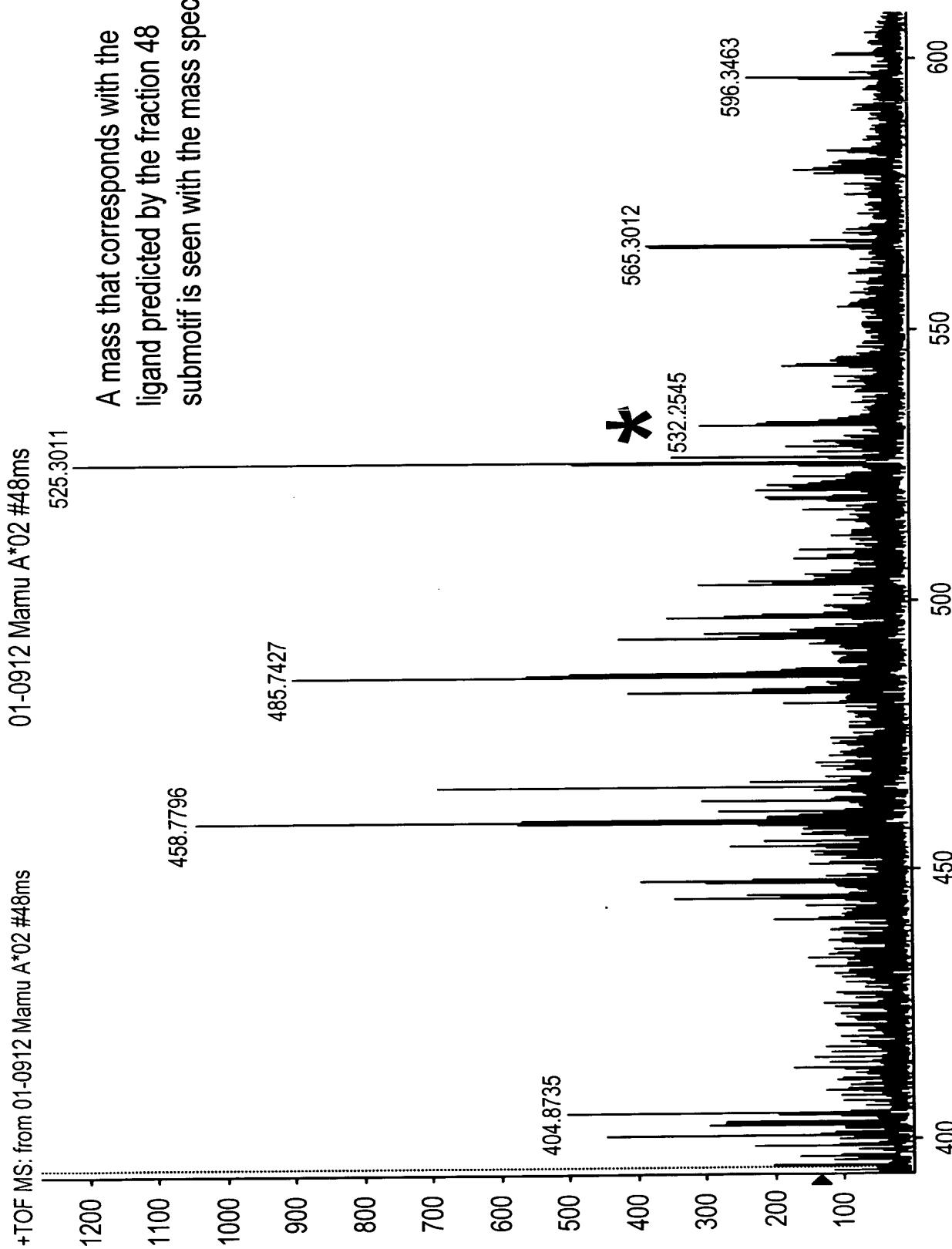


Fig. 9 m/z, amu

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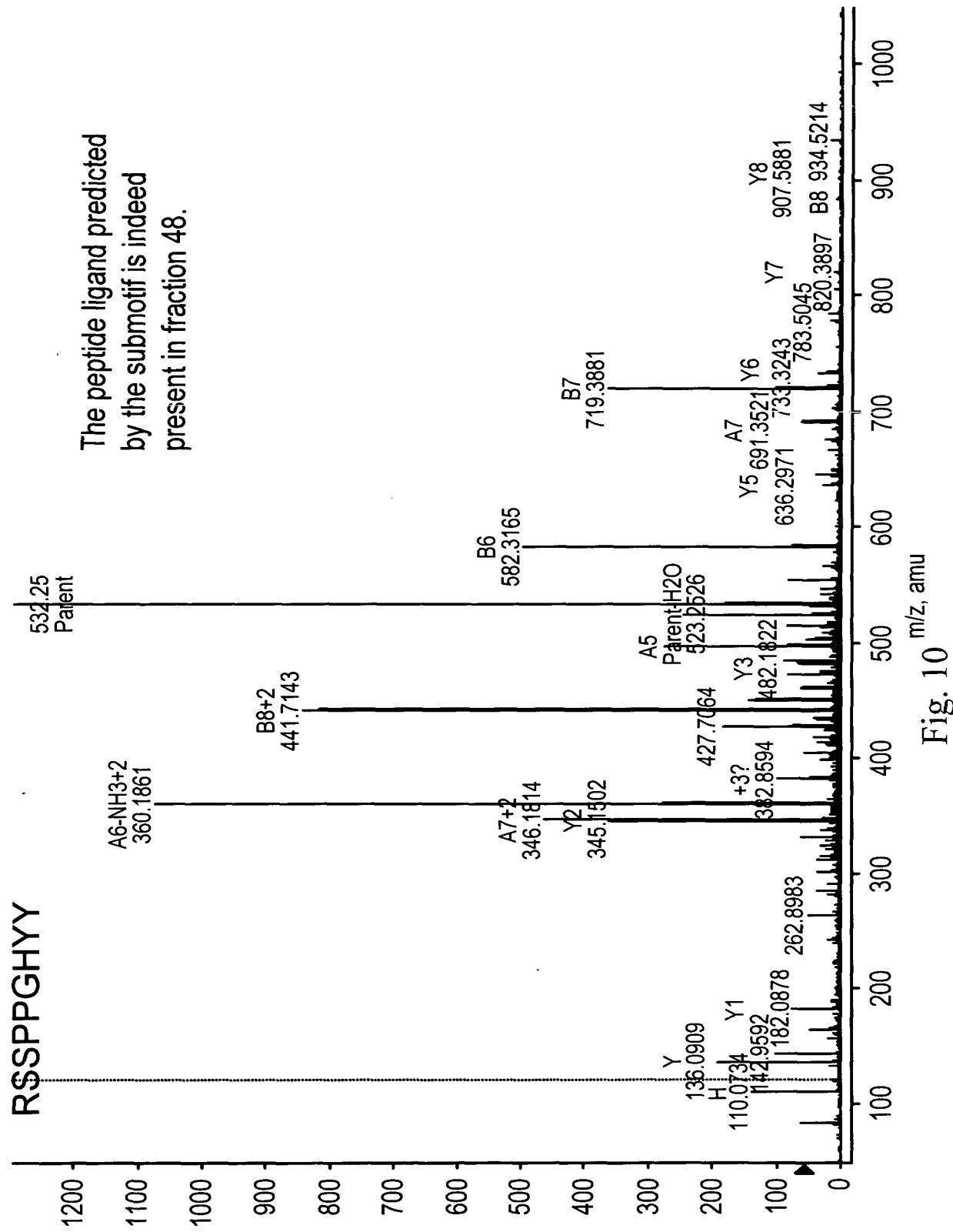


Fig. 10 m/z, amu

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Motif Data (Edman sequencing)

	1	%	fold	2	%	fold	3	%	fold	4	%	fold	5	%	fold	6	%	fold	7	%	fold	8	%	fold	9	%
Dominant							F	9.20	11.18																	
3.5 fold increases or more over prior round								7.60	7.01																	
								N	6.20	4.11																
								M	4.90	10.42																
Strong	K	31.50		R	53.80	2.57	C	8.20	3.10	P	8.30	2.97														
2.5-3.5 fold increase over prior round	R	15.50					K	5.40	2.67																	
	S	10.40					I	5.10	3.47																	
Weak							A	5.80	2.08				M	4.90	2.28											
2.0-2.5 fold increase over prior round																										
Trace							Q	3.60	1.79	P	2.20	1.94	K	11.00	1.63											
1.50-2.0 fold increase over prior round													S	6.00	1.68											
													V	5.30	1.99	H	1.80	1.67								

Fig. 11

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Design of HLA Ligand/Motif Database

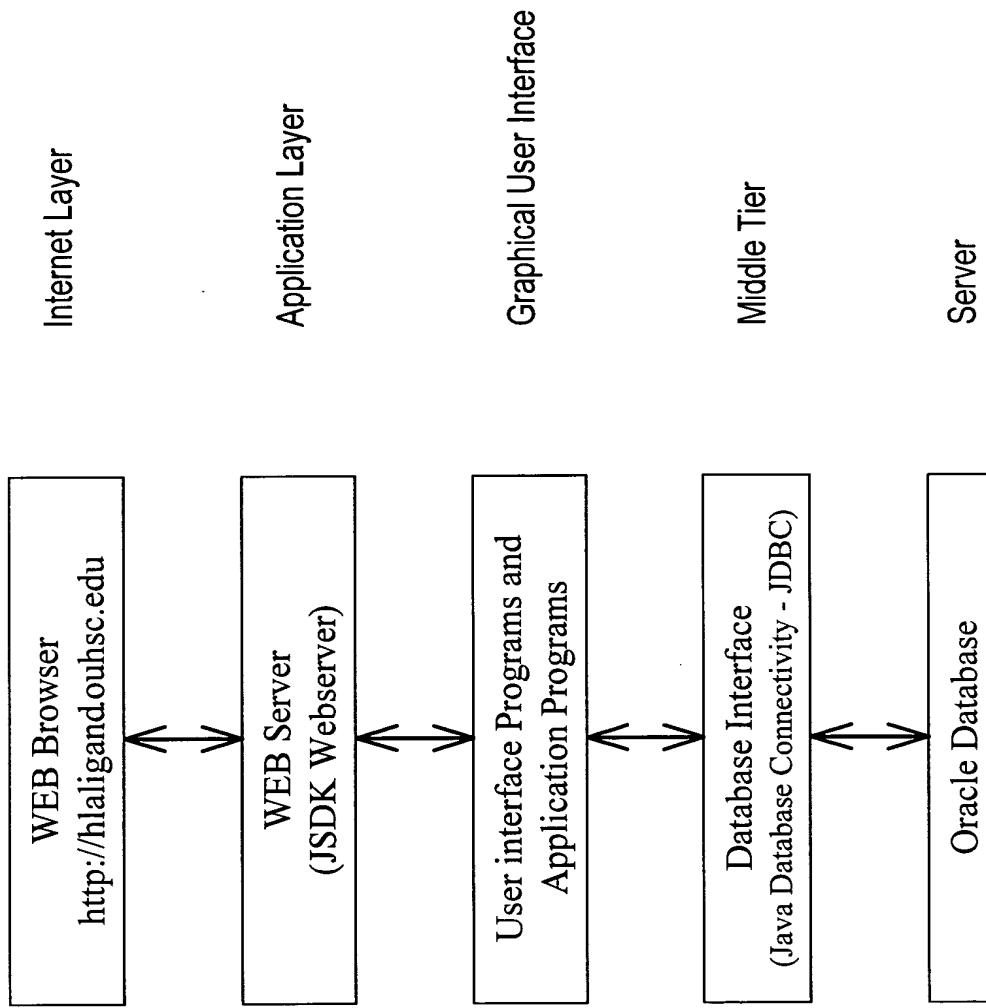


Fig. 12

Entity-Relationship (ER) Diagram for HLA Ligand/Motif Database

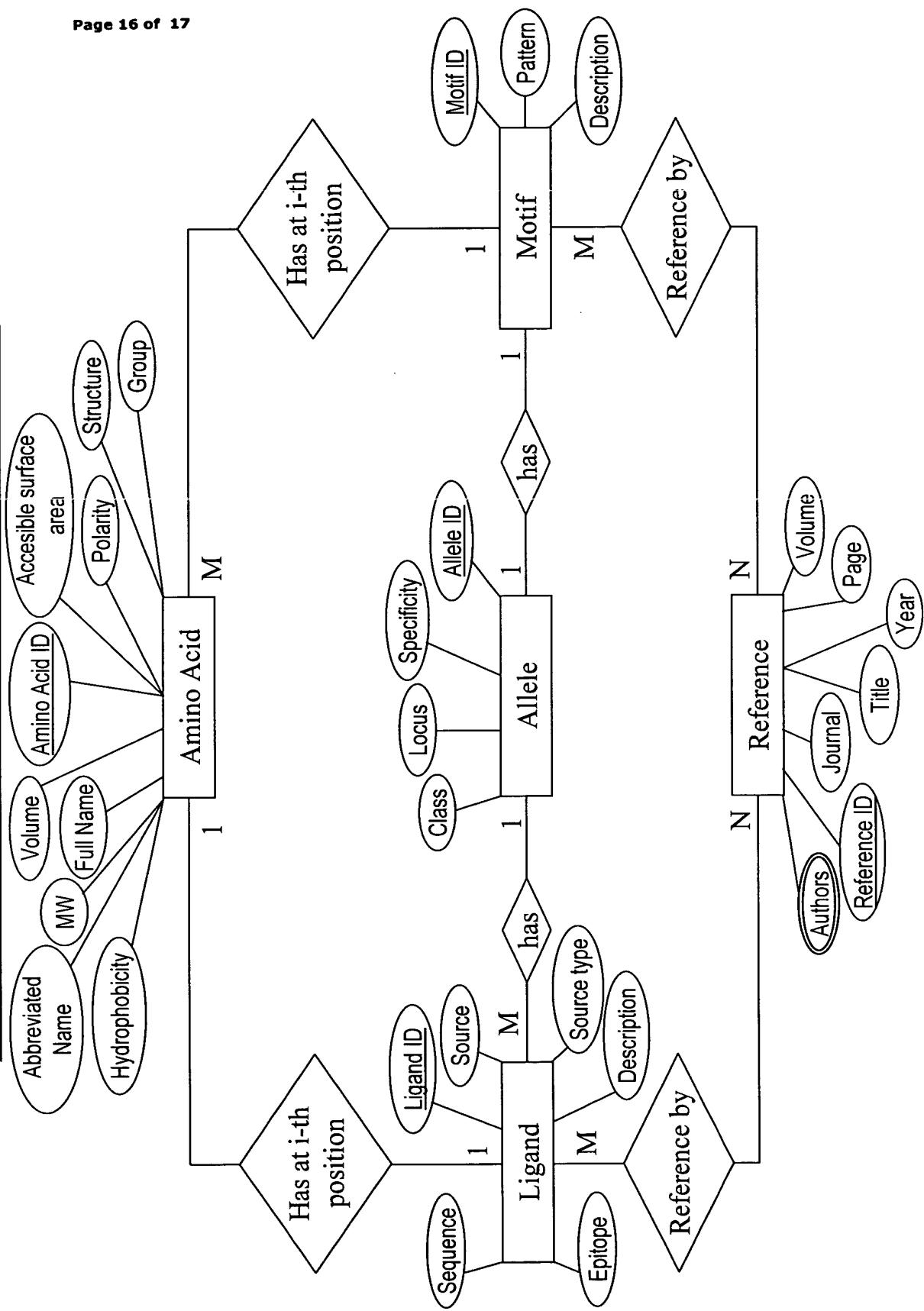


Fig. 13

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UML Diagram for HLA Ligand/Motif Database

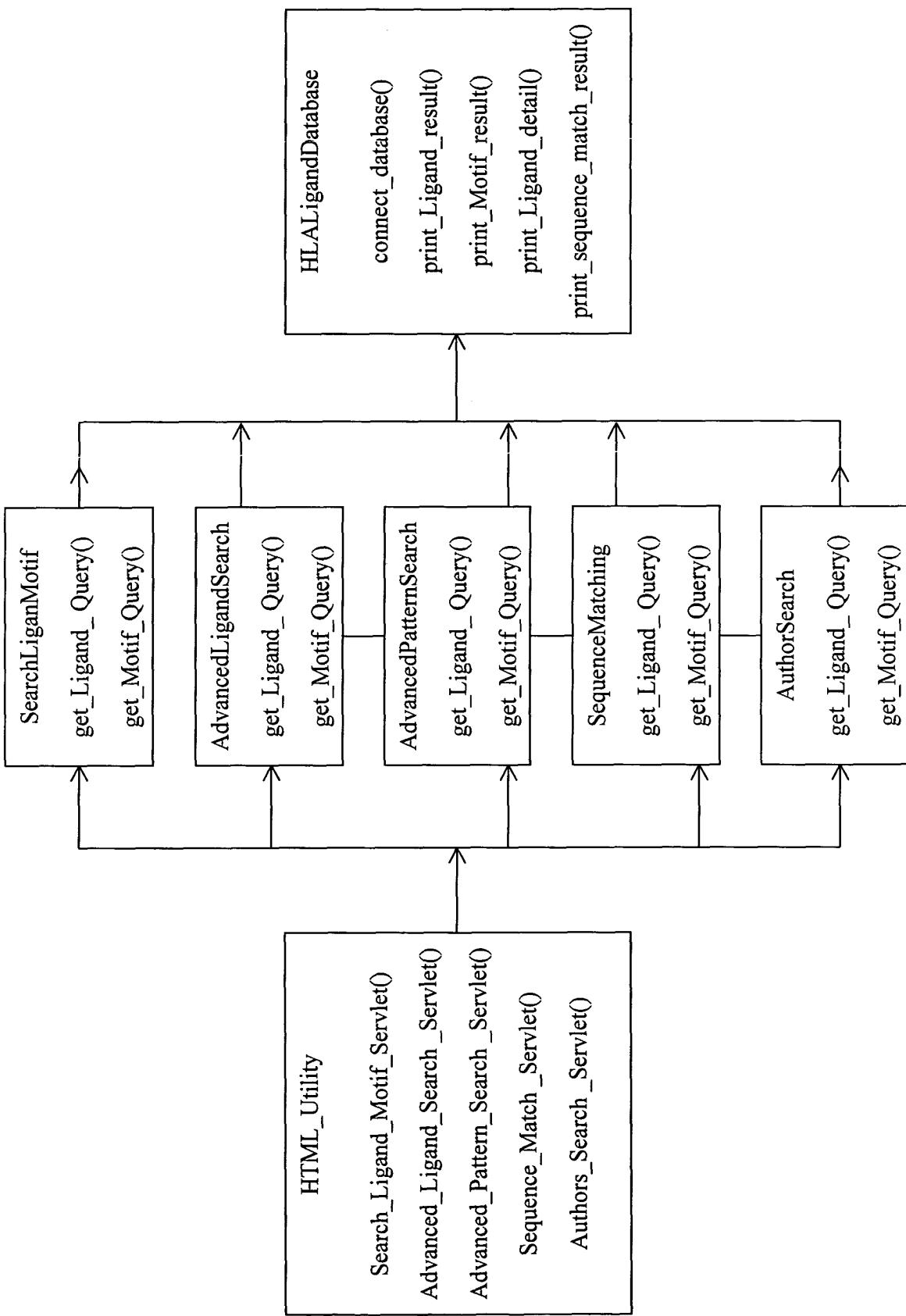


Fig. 14